REPORT

Type II collagen degradation and its regulation in articular cartilage in osteoarthritis

A R Poole, M Kobayashi, T Yasuda, S Laverty, F Mwale, T Kojima, T Sakai, C Wahl, S El-Maadawy, G Webb, E Tchetina, W Wu

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The progressive degeneration of articular cartilage is an underlying problem in the pathogenesis of osteoarthritis (OA) as well as in rheumatoid arthritis (RA) and other inflammation arthritides. It leads to a loss of joint function, frequently accompanied by debilitating pain.

In idiopathic OA this is a degenerative process that may cover a period of 20–30 years, culminating in clinical presentation and a need for joint replacement as the only effective means of managing this condition. There are as yet no recognisable disease modifying treatments for OA; only symptomatic treatment (pain relief) is possible.

The physical and economic burden of OA is enormous, affecting up to 15% of the total population (>50% of the aging population over 60 years of age). We face an enormous challenge in the management of this condition. Yet with recent progress in reaching an improved understanding of the pathobiology of this condition there is a realisation that there are recognisable targets for treatment. With the advent and promise of new methodologies to detect early disease and predict its progression, there are new opportunities for its future management.

In this paper we review what we know about the pathobiology of OA and how it can be investigated, focusing on the chondrocyte and the collagen fibrils that it produces which form the endoskeletal backbone of the extensive extracellular matrix.

STRUCTURE AND ORGANISATION OF ARTICULAR CARTILAGE

Articular cartilage contains only one cell type, the chondrocyte. In the adult this occupies <5% of the cartilage volume: the remainder is occupied by an extensive extracellular matrix. The structural backbone of this matrix is the collagen fibril (fig 1). This is composed mainly of type II collagen. It also contains type IX collagen on the surface of the fibril.[1] Immunological methods have been developed to determine the contents and measure the turnover of these molecules.[2] These fibrils also contain type XI collagen, both within and on the surface of the fibril and, as well leucine-rich proteoglycans, including decorin, fibromodulin, and biglycan.[1] Decorin and biglycan are most concentrated at the articular surface[3] where collagen fibrils are polarised in their alignment being parallel to the articular surface. This is where the tensile forces of shear are maximally developed as part of articulation and where the tensile strength of the cartilage is at its greatest.[1]

Between the collagen fibrils and in association with them are many other matrix molecules, the most common and largest of which is the large proteoglycan aggrecan. This forms macromolecular aggregates with hyaluronic acid which interact with the collagen fibrillar network[1] (fig 1). Aggrecan contains very many chondroitin sulphate and keratan sulphate chains which are heavily negatively charged because of the carboxyl and sulphate groups decorating these glycosaminoglycans. These glycosaminoglycan chains serve an extremely important function in that they bind large amounts of water and thereby create, with the containment of the collagen fibrillar network, a swelling pressure causing a compressive stiffness that resists deformation and compression of cartilage. Aggrecan is present at only low concentration at the articular surface and increases in content with depth.[1]

AGING OF ARTICULAR CARTILAGE AND THE DEVELOPMENT OF OA

The tensile properties of articular cartilage reach a peak at about 30 years of age.[4] Thereafter they deteriorate progressively in joints such as the knees and hips. The ankle shows much less evidence of age related degeneration and the development of focal OA lesions is much less pronounced. Aging modifies the collagen fibrils with the accumulation of non-enzymatic glycation end products that increase the stiffness of the collagen network.[5] Molecular analyses of aging healthy ankle articular cartilage have shown no major changes in the denaturation and cleavage of collagen by proteinases, nor in the synthesis of type II collagen and the content of the proteoglycan aggrecan.[6]

With immunohistochemistry of femoral condylar cartilages it is apparent that in aging the synthesis and cleavage of type II collagen is no longer confined to pericellular sites where matrix turnover is usually concentrated up to the age of about 30–35 years.[7 8] Instead we see evidence of collagen damage at and close to the articular surface, extending with age deeper into the cartilage and out into the territorial and interterritorial sites, remote from chondrocytes. Here in the same sites there is also evidence of matrix synthesis, not seen in younger cartilages (Poole AR, Pidoux I, unpublished data).[8] What is also interesting is that often we see a clear cut demarcation between this zone of "damage" to collagen fibrils and the more healthy cartilage deeper down.[8 9] Although changes occur with age, there is no obvious change in these matrix molecules when they are examined analytically in extracts of ankle articular cartilage throughout the depth of the cartilage.[6]

The first sign of overt degeneration characteristic of OA is the onset of fibrillation at the surface of articular cartilage as collagen fibrils are damaged. The proteoglycans decorin, biglycan, and aggrecan are lost and contents increase deeper in the cartilage as if to compensate for the more superficial loss.[3] A measurable increase in type II collagen denaturation is seen in early OA with a net loss of this molecule accompanying this damage.[10] This is associated with increased cleavage of the collagen by collagenases.[11 12] This is accompanied by an increase in the synthesis of matrix molecules, including type II

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Abbreviations: IL, interleukin; JSN, joint space narrowing; MMP, matrix metalloproteinase; OA, osteoarthritis; RA, rheumatoid arthritis, TGFβ, transforming growth factor β; TNF, tumour necrosis factor
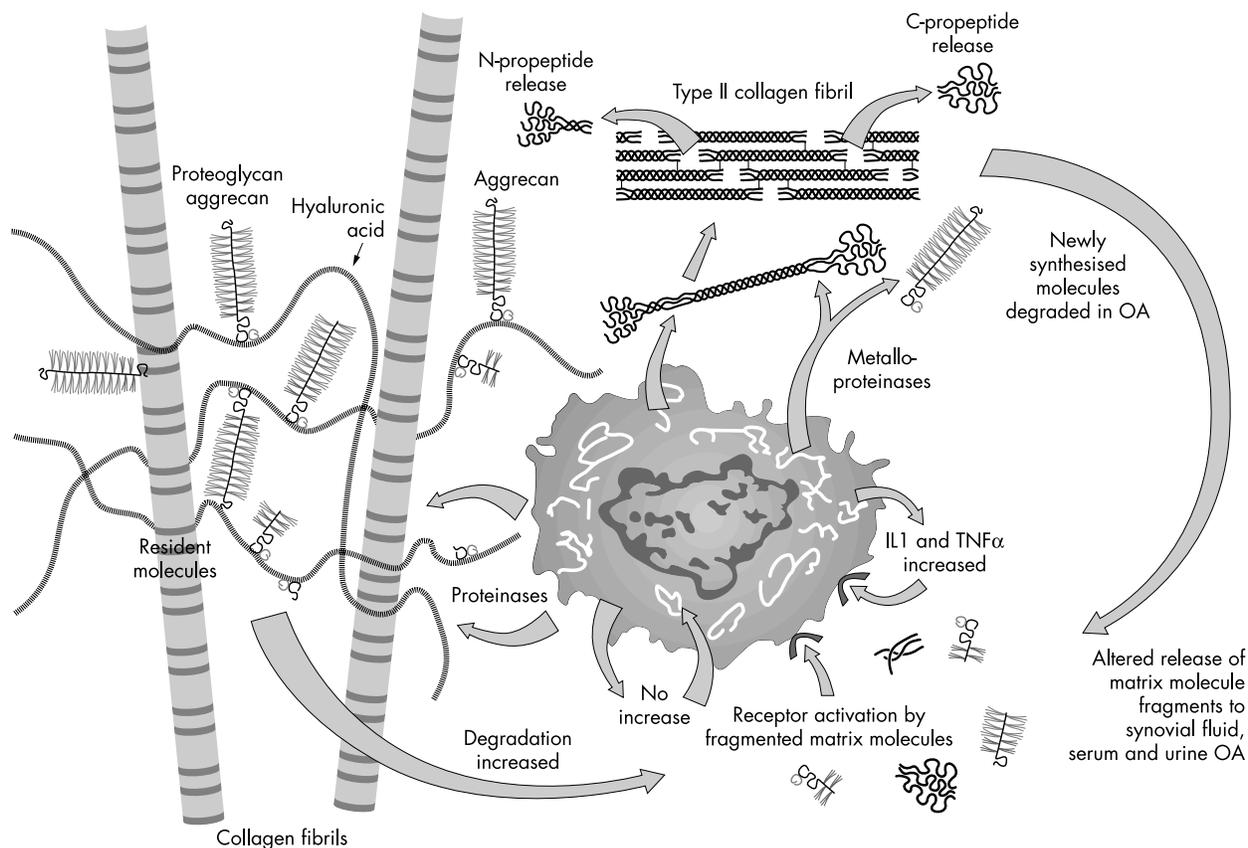
Figure 1 Diagrammatic representation of some of the elements of cartilage structure and matrix turnover. Type II collagen fibrils form an endoskeleton and associated with them in interfibrillar sites resides the proteoglycan aggrecan aggregated with hyaluronic acid. In physiology there is active pericellular turnover of extracellular matrix involving proteolysis mediated by matrix metalloproteinases (MMPs). In OA there is increased damage to resident matrix molecules more remote from chondrocytes with up regulation of synthesis of collagen and proteoglycan. These new molecules are also subject to degradation. Degradation products are released to body fluids where they can be detected. Increased proteolysis involves up regulation of interleukin (IL)1 and tumour necrosis factor β (TNFβ) expression by chondrocytes, nitric oxide (NO) production, and receptor activation by matrix degradation products, which is IL1 and TNFα dependent. Reproduced with permission from references 35 and 36.

collagen[8] and aggrecan.[1 13] But the newly synthesised molecules are often damaged,[12 13] compromising any effective attempts at cartilage matrix repair. Early but limited proliferation of chondrocytes is restricted by the physical bulk of the large damaged collagen fibrils that occupy much of the extracellular matrix. Other than the chondrocytes, there is no machinery to remove and repair this damaged tissue because mature articular cartilages is avascular. Only in advanced disease is some local vascular invasion seen at the cartilage-bone interface.

The degradative changes involving collagen accompany the loss of proteoglycan, all of this usually starting at the articular surface.[7 9] Studies with mice expressing a regulatable active human collagenase (MMP-13) transgene[14] have shown that extensive damage to collagen and aggrecan occurs, leading to cartilage thinning, before overt fibrillation is seen (Wahl SL *et al*, unpublished data). Focal lesion development is seen at sites of direct mechanical contact between opposing articular surfaces in the knee.[14] This suggests an important role for local mechanical forces acting to cause the development of overt focal lesions where cartilage is lost, eventually leading to eburnation of calcified cartilage and bone.

Changes in mechanical loading of chondrocytes caused by the damage to and loss of matrix molecules no doubt contribute significantly to the degenerative pathology because it is well known that abnormal excessive and non-cyclic loading can stimulate cartilage degeneration in vitro.[1] Altered loading after joint injury to cruciate ligaments or menisci, or both, also leads to a rapid onset of abnormal cartilage matrix turnover,

commonly culminating in clinical post-traumatic OA. In rabbits, as in mice and rats, these focal lesions develop either as a result of joint instability induced experimentally or naturally as in human aging.[15 16]

Associated with these lesions we see clear evidence of increased damage to adjacent cartilage with increases in type II collagen denaturation, cleavage, and a loss of collagen content accompanying that of aggrecan (Webb G and Poole AR, unpublished data). These changes suggest that idiopathic OA can develop from focal lesions, which progressively enlarge in size.

CELLULAR AND DEGRADATIVE MECHANISMS THAT CAUSE CARTILAGE DEGENERATION

A considerable amount of work in recent years has centred on reaching an improved understanding of the mechanisms whereby cartilage is resorbed in arthritis. There is a clear consensus that MMPs have a key role in the degradation. There are 27 MMPs at a recent count. Of the "classic" collagenases there are four—namely, MMP-1 (interstitial collagenase), MMP-8 (polymorph collagenase), MMP-13, and MMP-14 (membrane type I MMP), which seem to be more "dangerous" than the others. MMP-13 is probably the collagenase which plays the greatest part in the pathology of OA degrading the "resident" collagen fibrils more remote from the cell in the territorial and interterritorial matrix.[12] This is the same collagenase that is used to remodel matrix in the growth plate.[2 17] MMP-1 may be more important in a minority of patients and is believed to be more involved in the degradation of newly

synthesised collagen.[12] The activities of collagenases are clearly increased in both advanced and end stage OA[11 12] and in the early development of focal osteoarthritic lesions (Webb G and Poole AR, unpublished data).

Aggrecan is cleaved both by different MMPs and also by aggrecanases at distinct sites in the core protein.[18] There is evidence for the activities of both types of proteinases in OA cartilage.[19] It is unclear how important the aggrecanases are at this time. Even if they are blocked therapeutically we have no indication that this will prevent aggrecan degradation even in culture (in view of alternative pathways and proteinases for cleavage), whereas in the case of the collagenases we know that their inhibition can measurably reduce collagen degradation in culture[12] and in vivo.

After the initial cleavage of type II collagen by collagenases it is denatured and lost. Chondrocytes subsequently undergo further phenotypic change becoming hypertrophic and expressing and secreting type X collagen.[20] This differentiation is normally seen in the growth plates as part of endochondral ossification.[1 2 17] Cleavage of type II collagen by MMP-13 is required for this differentiation of chondrocytes and matrix mineralisation.[17] Calcification of articular cartilage matrix also occurs in OA in association with these changes.[20] Thus chondrocyte differentiation in OA seems to be a response to extensive damage to the collagen fibrillar network, and collagenase activity seems to play an important part in this. What is of concern is that these hypertrophic cells also up regulate expression of MMP-13 and other MMPs, including the gelatinase MMPs 2 and 9.[1 16] Moreover, hypertrophic cells eventually undergo apoptosis.[1] This is commonly seen in OA cartilage.[21]

Thus the cartilage in OA is undergoing a process of chronic degenerative change compounded by phenotypic changes associated with chondrocyte differentiation. The damage to the collagen, its loss with that of aggrecan, and matrix calcification create a poor alternative to the robust strong and remarkably resilient cartilage found in non-arthritic subjects. It is under these conditions, in particular, that mechanical loading more directly traumatises and damages the cartilage, leading to a net loss of cartilage.[22]

What are the chemical signals that are driving this process? During matrix degradation a wide variety of molecular fragments are produced as a result of the extensive proteolysis referred to above. Some of these are known to induce the expression and secretion of MMPs and prodegradative cytokines such as IL1 and TNFα.[20] These include fibronectin and type II collagen fragments.[20] Whereas fibronectin fragments from different regions of the molecule can induce both the degradation of collagens and aggrecans, a type II collagen fragment from the helical region of this molecule can only induce collagenase activity as well as the above cytokines (Yasuda T et al, unpublished data). These fragments engage specific receptors which include the integrins $\alpha_5 \beta_1$ in the case of fibronectin receptor ligand engagements. These activate specific protein kinases, which induce an up regulation of MMPs and at low concentrations may induce matrix synthesis as in the case of fibronectin fragments.[23] The activation of chondrocytes by these matrix degradation products involves the expression of IL1 and TNFα (fig 1). Inhibition of either cytokine can often arrest the resultant degradation of extracellular matrix (Kobayashi M et al, unpublished data).

These cytokines appear to have a key role in cartilage degeneration in OA. Not only are these cytokines up regulated in chondrocytes but so also are their receptors.[20] Inhibition of IL1 activity can arrest production of nitric oxide, a damaging free radical.[24] Both IL1 and TNFα are actively generated by chondrocytes in a majority of patients. That they are directly involved in the cleavage of collagen and proteoglycan is shown by the ability to block matrix degradation in cultures of OA cartilage using IL1 receptor antagonist protein (IL1Ra) and a soluble TNFα p55 (type I) chemically modified receptor protein. This leads to the arrest of the excessive cleavage of

collagen by collagenase in two thirds of patients studied and an arrest of degradation and increase in aggrecan content in about half these patients (Kobayashi M and Poole AR, unpublished data). Thus IL1 and TNFα each represent potentially very important therapeutic targets, not only in RA but also in OA, for the control of the degradation of articular cartilage in arthritis. The transforming growth factor βs (TGFβ) are also extremely important in regulating cartilage turnover and maintaining a healthy cartilage. Thus in mice in which the TGFβ type II receptor[25] or the TGFβ driven SMAD signalling pathway[26] have been made defective the articular cartilage degenerates. In general, the production and concentrations of different cytokines and proteinases that are released from chondrocytes are critically important in the physiology and pathology of cartilage.[27]

SURROGATES FOR THE DETECTION OF JOINT DEGENERATION AND ITS PROGRESSION IN OA

Some significant advances have been made recently in our ability to measure early changes in articular cartilage that herald developing pathology.

The use of magnetic resonance imaging in conjunction with the contrast medium gadolinium permits early detection of a loss of aggrecan because the normal content of aggrecan in cartilage ordinarily excludes gadolinium from the extracellular matrix.[28] This technology offers much promise in the detection of early disease and in studying its progression.

The use of biomarkers seeks to identify process rather than outcome, which may be predictable if the process leads to a defined clinical outcome such as joint space narrowing (JSN), our poor but only "gold standard" that is used to assess disease progression in OA. Because routine radiology to establish JSN is fraught with problems in accurately reproducing measurements, measurable change may require clinical trials of 2–3 years' duration, with very large numbers of patients, and the requirement for new predictors or surrogates of clinical outcome remains a major unmet need in drug development in OA. Fortunately, recent developments hold much promise for the future. Immunoassays have been developed to detect cleavage products of type II collagen in body fluids such as serum and urine.[29] The synthesis of type II procollagen can be detected by measurement of propeptides released from this molecule as it is incorporated into collagen fibrils in the matrix.[8] Recent studies involving combinations of degradation (C-telopeptide assay) and synthesis markers and degradative markers (collagenase cleavage epitope assays) have shown that it is now possible to predict clinical outcome from the JSN over a period of 1–2 years (Cerejo R, et al, unpublished data).[30 31] We can use statistical analyses to distinguish subgroups of patients with large joint disease where joint degeneration may progress rapidly or slowly. These new developments offer much promise for the future management of clinical trials whereby drug treatment for one to two months may lead to a change in synthesis and/or degradation, which is measurable and predictive of outcome over one to two years. Such opportunities will negate the need for the traditional clinical trials once these studies have been confirmed and validated with careful clinical assessments. The introduction of these new biomarkers into clinical trials in the immediate future should help to remove the barriers to drug development created by the enormous financial burden of the traditional OA clinical trial.

Biomarkers also offer opportunities to study arthritic disease activity in vivo (both OA and RA) and its treatment. Examples of this for the collagen fibril in cartilage are seen in preclinical studies of collagen[32 33] and aggrecan[34] degradation. Biomarkers can be used to set doses of drugs to avoid impacting significantly on normal skeletal turnover. They can also be used to screen populations of normal people, where they may be stratified and classified by their biomarker levels. This may

be of help in identifying genetic-biomarker linkages, patients at risk for OA, and the establishment of more uniform biomarker defined patient populations for clinical trials involving these new technologies.

CONCLUSIONS

This overview of some of the work of this and other laboratories has sought to capture some of the recent developments in our understanding and management of the pathobiology of OA. New therapeutic targets have been identified. In vivo assessment of disease activity and progression is now a more realistic opportunity than a couple of years ago. The immediate future should herald some major advances in creating and assessing the first disease modifying treatments for OA.

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Authors' affiliations

A R Poole, M Kobayashi, T Yasuda, S Laverty, T Kojima, T Sakai, C Wahl, S El-Maadawy, E Tchetina, Shriners Hospitals for Children, Departments of Surgery and Medicine, McGill University, Montreal, Quebec, Canada
M Kobayashi, T Yasuda, Department of Orthopaedic Surgery, Kyoto University Medical School, Kyoto, Japan
S Laverty, Faculty of Veterinary Medicine, Department of Clinical Sciences, University of Montreal, Ste-Hyacinthe, Quebec, Canada
F Mwale, Department of Orthopaedic Surgery, Jewish General Hospital, Montreal, Quebec, Canada
T Kojima, Department of Orthopaedic Surgery, Nagoya University School of Medicine, Nagoya, Japan
G Webb, Molecular Cell Biology Unit, Glaxo Wellcome Research, Stevenage, Hertfordshire, UK
W Wu, Arthritis Unit, Massachusetts General Hospital, Boston, MA, USA

Correspondence to: Dr A R Poole; rpoole@shriners.mcgill.ca

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